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Effect of Intensive phototherapy on immune system cells in Newnatal Jaundice

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ABSTRACT

Background: Neonatal jaundice is one of the most common conditions challenging neonatologists daily is usually used for treatment.In this study, we aimed to assess the role of intensive phototherapy on T & B lymphocyte in neonatal jaundice by measuring the percentage of CD19+, CD4+ and CD8+ lymphocytes subsets in newborns with indirect hyperbilirubinemia both; before and after 72 hours from exposure to the intensive phototherapy including the treatment effects on the part of the immune system. **Patients and methods:**A prospective cohort study was carried out in Zagazig University Hospital, PediatricPediatric neonatal intensive care unit (NICU) and Clinical Pathology departments.Included 25 term newborns with indirect hyperbilirubinemia admitted to the Neonatal Intensive Care Unit and exposed to phototherapy for treatment, and the control group had 25 healthy term newborns.The percentages of CD19+, CD4+, and CD8+ lymphocytes in the peripheral blood were measured in the samples using immunophenotypic analysis by flow cytometry. **Results:**There is a significant difference regarding total bilirubin and indirect bilirubin before and after phototherapy. There is no significant difference regarding CD4, CD8 and CD19 for the patient group. Furthermore, the study showed that the percentages of CD4+ and CD8+ lymphocytes subsets showed no change in newborns after 72 hours of exposure to phototherapy, but CD19+ was highly significantly lower at before but no significant difference at after.**Conclusion:**this study demonstrated that phototherapy did not affect either B and T cells or the occurrence or rate of infection and need for hospitalization.

Keywords: Intensive phototherapy**,** T & B Lymphocyte, Neonatal Jaundice

INTRODUCTION

Neonatal jaundice occurs in about 50-60% of full-term newborns and 80% of premature infants, and it is more pronounced by day 3-4 of their life. Hyperbilirubinemia attracts the attention of specialists not only due to its high frequency among newborns but also because a high bilirubin level can become dangerous for a baby, causing both morbidity and mortality**[(1)].**

Although a significant amount of ultraviolet (UV) light is absorbed in the epidermis, a small but essential part passes through the epidermis and reaches the dermis. In this way, T

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lymphocytes located around the capillaries of the papillary dermis and T-lymphocytes in the post capillary venules of the papillary dermis may be exposed to low dose UV light[(2)].

Phototherapy, a non-invasive readily available therapy, has been widely used to treat neonatal jaundice for more than half a century. It effectively treats neonatal unconjugated hyperbilirubinemia at wavelengths 425-475 nm[(3)].

Both intensive and conventional phototherapy cause endogenous mononuclear leukocyte DNA damage in the same way and same percentage in jaundiced term infants, and thus it might affect the immune system[(4)]. This study aimed to investigate intensive phototherapy's effect used in the treatment on neonatal hyperbilirubinemia on B and T lymphocyte subsets by measuring the percentages of CD4+, CD8+ and CD19+ lymphocytes as a reflection of the immune status of the neonates, then the first 6 months of life.

Patients AND METHODS

A prospective cohort study was conducted on 50 full-term newborns were divided into 2 groups of age and matched sex; Control Group: Twenty-five matched healthy full-term newborns without neonatal jaundice nor received phototherapy. Patients Group: Twenty-five full-term newborns with neonatal indirect hyperbilirubinemia treated by intensive phototherapy for an average of 3 days. Oral consent was obtained from the parents for every neonate to meet standard patient's rights.

Inclusion criteria were both male and female. Full-term neonates with > 2.5 kg. Neonate's age ranges from 1 to 28 days old at the time of admission with indirect hyperbilirubinemia in need of phototherapy. Postnatal age less than or equal to 14 days, with neonatal indirect hyperbilirubinemia and treated by conventional phototherapy based on American Academy of Pediatrics recommendations. Patients Group full-term newborn of gestational age of more than 37 weeks. Untreated control group: healthy full-term newborn without neonatal jaundice both clinical and laboratory.

All patients were subjected to full history taking, full clinical examination. Phototherapy was carried out for the patients' group as the infants were uncovered except for shielded genitalia and eyes. White fluorescent lamps emit light at a wavelength of 420-470-nm, placed at a 40 cm distance from the neonates.

Blood sampling:

Follow-up with the diseased group is needed for six months after exposure. Seventy-five blood samples were collected from both; patients' group and the untreated control group. For the patient's group, 25 samples were taken before phototherapy, while the other 25 were taken after 72 hours of exposure to phototherapy. The rest of the samples refers to the untreated control group. Besides, for immunophenotypic analysis, 2ml of blood was collected in vacutainers tubes containing ethylene-diamine-tetra-acetic acid and thus analyzed by flowcytometry within 24 hours after collection to test whether there were any deviations in the proportions of CD4+, CD8+ and CD19+ lymphocytes in peripheral blood.

Follow up:

Clinical follow up of patients group for a period of six months after phototherapy to monitor any incidence, frequency of infection or need for hospitalization. Some patients had

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upper respiratory tract infections in the form of fever, cough, and runny nose, while some patients had gastroenteritis in the form of diarrhoea. Most respiratory virus infections in early childhood are confined to the upper respiratory tract, Where Upper respiratory tract infection (URTI) in infants may lead to lethargy and poor feeding. Infective gastroenteritis in young children is characterized by the sudden onset of diarrhoea, with or without vomiting. Most cases are due to a viral infection, but bacterial or protozoal infections cause some. The illness usually resolves without treatment within days, but severe diarrhoea can rapidly cause dehydration.

Statistical Analysis

All Data out of samples were collected, tabularized and statistically analyzed. Before statistical analysis, Data collected throughout history taking, essential clinical examination, laboratory investigations and outcome measures were coded, logged and analyzed using Microsoft Excel software. Statistical analysis was conducted according to the type of data qualitative represent as number and percentage, quantitative continues group represented by mean ± SD. The following tests were used to verify the differences for significance; difference and association of qualitative variable by Chi-square test (X2) and differences between quantitative independent groups by T-Test or Mann Whitney, paired by paired t or Sign (T. Johnson, 2013). The Chi-square test was used to compare qualitative variables between groups. Mann-Whitney test was used to compare two medians and two ranges of two groups. The level of significance: P>0.05 means no significance, P<0.05 means significant and P<0.01 means highly significant.

RESULTS

This table showed a statistically significant difference regarding total Bilirubin before and after table (1a).A significant difference regarding Indirect Bilirubin before and after table (1b).A significant difference regarding P-1 Total Bilirubin and P-2 Indirect Bilirubin before and after table (1c).

Table 1 (a): Bilirubin distribution among studied groups.

(a)	Control Group	Patient Group	T	P
T bilirubin [before] (mg/dL)	1.74±0.34	17.74±4.17	19.095	0.00**
T bilirubin [after] (mg/dL)	1.74±0.34	8.10±2.66	8.965	0.00**
P		0.00**		

Table 1 (b): Bilirubin distribution among studied groups.

(b)	Control Group	Patient Group	T	P
Indirect bilirubin [before] (mg/dL)	1.07±0.15	16.43±4.12	18.599	0.00**
Indirect bilirubin [after] (mg/dL)	1.07±0.15	6.30±2.08	9.123	0.00**
P		0.00**		

Table 1 (c): Bilirubin distribution among studied groups.

(c)	Control Group	Patient Group	T	P
P-1 T bilirubin (before & after)		0.00**		

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P-2 Indirect bilirubin (before & after)		0.00**		
P		0.00**		

Table 2; showed that no significant difference between groups; there was no significant change among patients before and after. That significantly lower than before, but there was no significant difference after, and the patient group significantly increased after treatment. The patient group was highly significantly lower than before, but there was no significant difference after and the patient group highly increased dramatically after treatment.

Table (2): CD4, CD8 , CD19 distribution before and after among studied groups

	Control Group	**Patient Group**	**Mann Whitney**	**P**
CD4_before	15.30 (2.3-21.6)	13.85 (0.6-44.8)	1.743	0.085
CD4_after	15.30 (2.3-21.6)	16.10 (3.8-50.9)	1.034	0.265
	-	0.058	-	-
CD8_before	12.20 (2.9-20.8)	7.90 (0.67-22.7)	2.269	0.032*
CD8_after	12.20 (2.9-20.8)	10.85 (2.11-23.1)	1.584	0.096
		0.042*		
CD19-before		8.60 (1.9-14.8)	2.27 (0.29-6.9)	
CD19-after		8.60 (1.9-14.8)	7.85 (1.78-12.65)	
		0.00**		

There was no significant difference between groups; also, there was no significant change among patients between before and after figure 1.

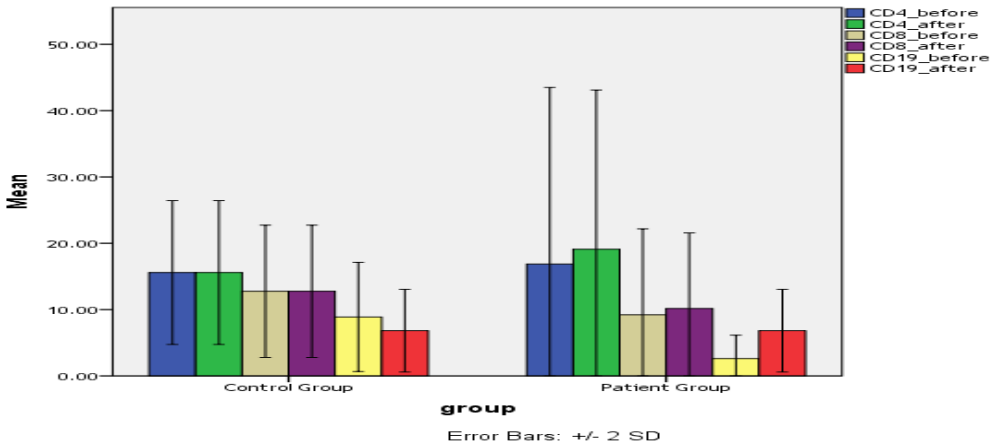

Figure (1): CD4, CD8, &CD19 distribution before and after among studied groups.

No significant correlation was detected with CD4 or CD8, but there was a significant negative correlation between CD19 before and after treatment and TSB figure 2.

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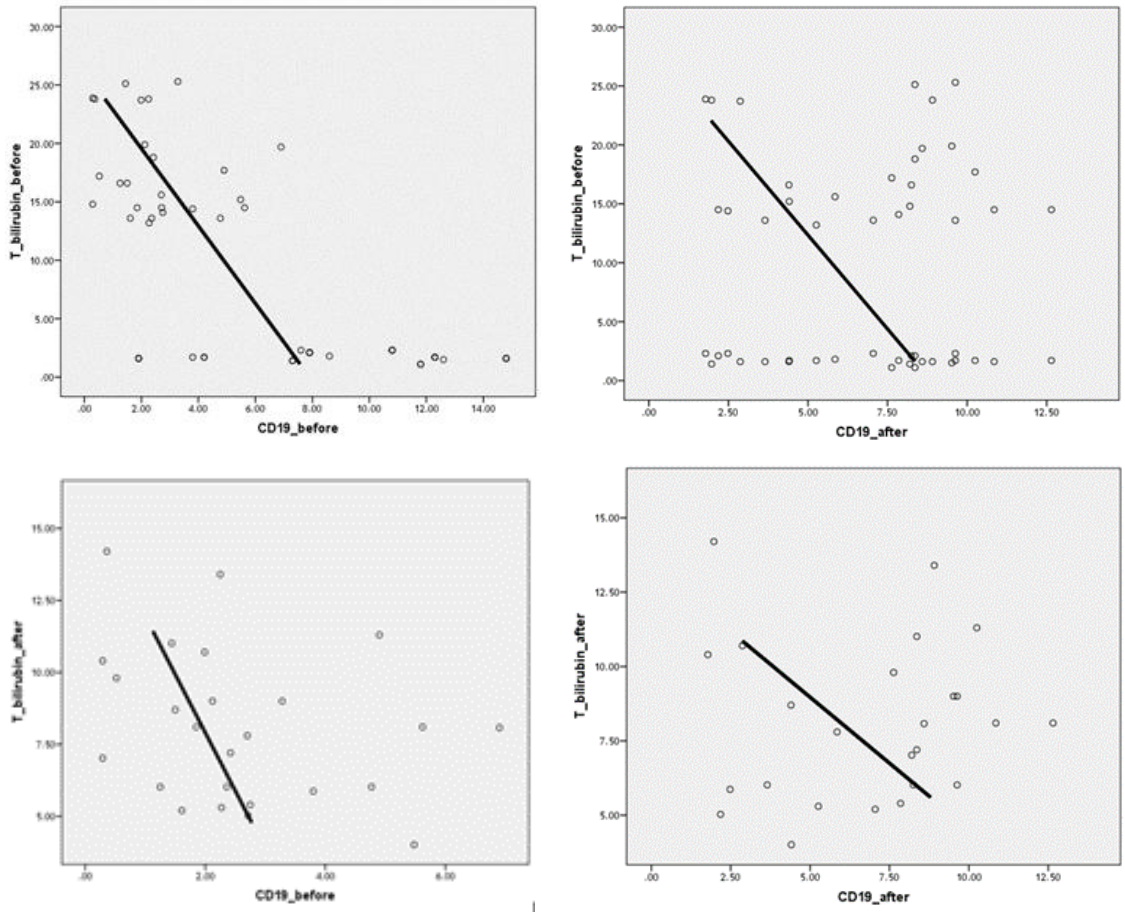

Figure (2): Correlation between TSB and percentages of CD19 before treatment.

Figure 3; showed that No significant increase in infection rate and hospital admission after 6 months in the following up in the studied cases.

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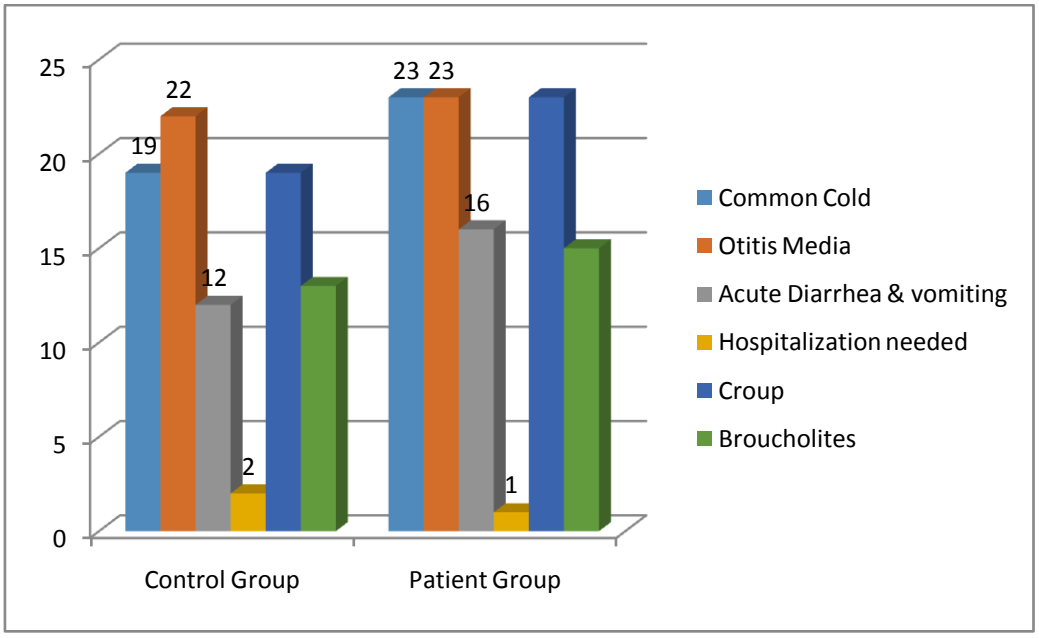

Figure (3): Follow up data distribution between studied groups.

Positive correlation between times of infection and value of CD4, CD8 Before only figure 4.

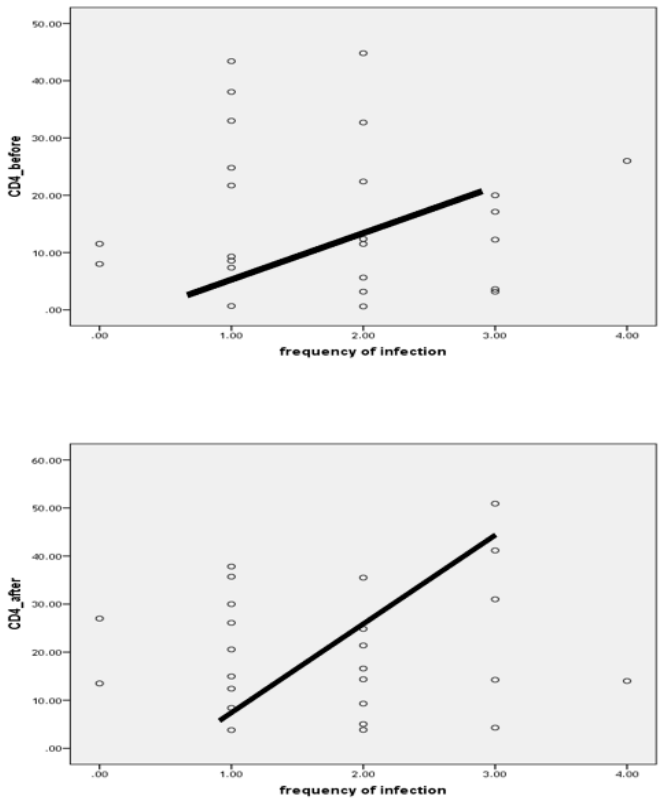

Figure (4): Correlation between frequency of infection and CD.

Figure 5; CD4 and CD8 were significantly higher among cases had infection.

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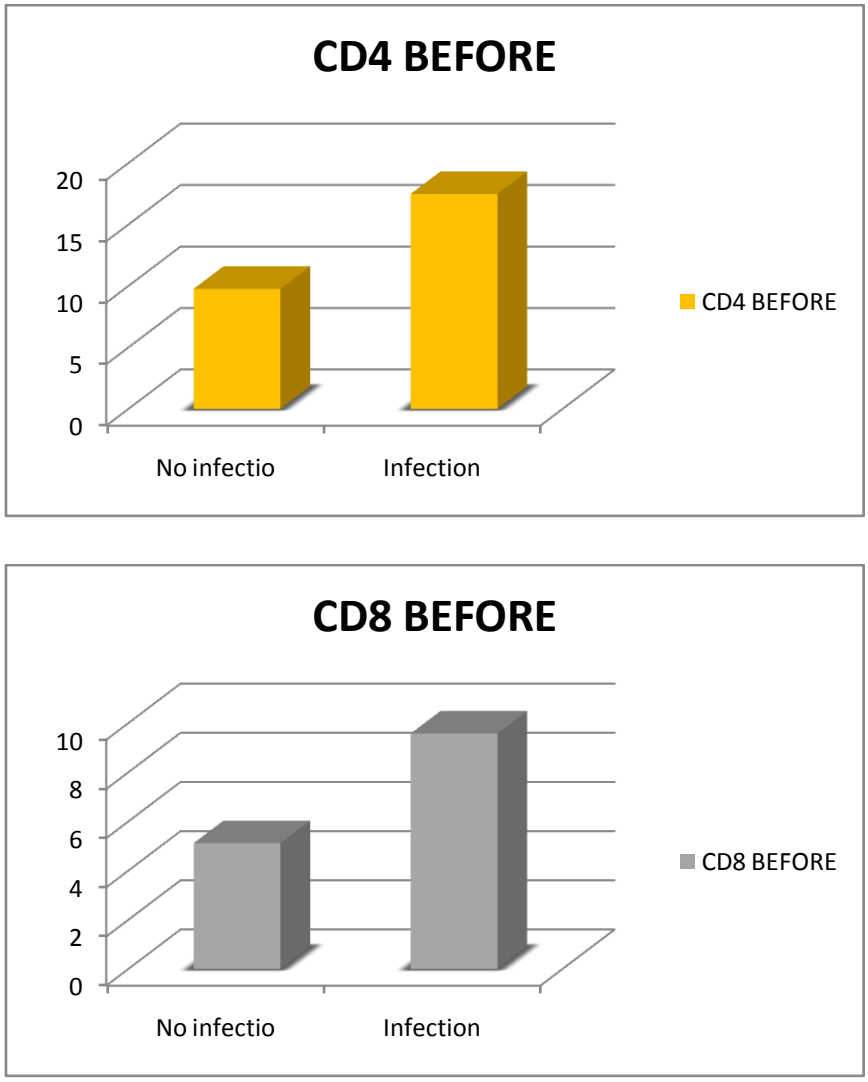

Figure (5): Relation between infection and CD.

DISCUSSION

From our study, there is a statistically significant difference regarding total bilirubin before and after among the patient group (17.74±4.17), (8.1±2.66) respectively, where P < 0.001).

In our study, a comparison between patients' total serum bilirubin before and after phototherapy showed a statistically significant difference ($p<0.05$), meaning that phototherapy has a positive effect on neonatal hyperbilirubinemia. These results go in agreement with**Yahia et al.** [(5)]**andShapiro & Riordan**[(6)],whom all found that ultra-violet phototherapy is a potent and effective treatment for neonatal jaundice.

This study showed that the percentages of CD4+ and CD8+ lymphocytes subsets showed no change in newborns after 72 hours of exposure to phototherapy, but regarding CD19 (B lymphocyte) in our study, there is statistically significant lower CD19 in patient group in comparation to control group before phototherapy but after phototherapy, there are no statistically significant difference.

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In our study, upon comparing patients before phototherapy and untreated control group as regard CD19+, CD4+ andCD8+ lymphocyte percentage, there was a statistical difference only in CD19+ lymphocyte percentage.

In our study, although CD19 lymphocytes percentage was significantly higher in the untreated control group than patients before phototherapy, there was no significant difference in CD19 between patients before and after 72 h. But still, there was an increase in the percentage of CD19 lymphocytes 72 h after phototherapy, which would be closer to the untreated control group. This increase was highly related to the TSB level, where a decrease in TSB level led to an increase in CD19+lymphocytes percentage. These results were in line with **Ventura et al.** [7], who demonstrated that unconjugated hyperbilirubinemia has an inhibitory effect on CD19 B cells, resulting from the induction of necro-sis apoptosis in mature immune cells.

In the present study, there was a significant correlation between total serum bilirubin level and CD19+lymphocyte % before and after phototherapy ($p<0.05$). These results are in line with **Khan and poduval**[8]**,** who demonstrated that unconjugated hyperbilirubinemia has an inhibitory effect on CD19 B cells, which could result from induction of necrosis apoptosis in mature immune cells.

Our results agree with**Rashedy et al.** [9]**,** who studied the effect of phototherapy on some lymphocytes subsets (CD4, CD8, CD19) in 30 term neonates with indirect hyperbilirubinemia and 25 healthy term neonates as a control group. They found no statistically significant difference between lymphocytes subsets before and after 72 hours of exposure to phototherapy.

These results agree with studies done by **Karabayir et al.** [10]and **Ebbesen et al.** [11], who reported similar results. Moreover, our results agreed with **Rashedy et al.** [9]. Who found that all lymphocyte subsets were not statistically significantly decreased by the 72h of exposure to phototherapy, except for the percentage of T lymphocyte subset, which was considerably lower in newborns at 72h of exposure tophototherapy.4Karabayir et al. noticed a significant increase in CD4+ rate after eight hours of phototherapy. However, there was no significant change in lymphocyte subsets 48 h after phototherapy.

Karabayir et al. [10] studied the effect of phototherapy on the CD4 and CD8 lymphocyte level in newborns using 22 term neonates with indirect hyperbilirubinemia and 25 control term neonates without hyperbilirubinemia. He found that apart from the significant increase was determined in CD4 ratios after eight hours of the phototherapy ($p< 0.05$), and non-significant change was determined in CD4 and CD8 lymphocyte level 48 hours after phototherapy ($p > 0.05$). This difference compared to our results may be due to the time of measurement for CD4 and CD8 after phototherapy.

There was no statistical difference in our study on comparing the ratio between CD4/CD8 ratio among the studied groups and patients before and after exposure to phototherapy. These results concurred with **Karabayir et al.** [10].who also found no change in

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CD4/CD8 ratio among patients or the studied groups. This study showed no significant difference in CD4 level and CD4/CD8 ratio among patients after phototherapy compared to control group but there is a significant difference in CD8 level among patients after phototherapy compared to the control group.

Ped et al. [12]studied the effect of phototherapy on the lymphocyte subsets in newborns among 22 term neonates with indirect hyperbilirubinemia and 25 control term neonates without hyperbilirubinemia. He noticed a significant increase in CD4+ % after eight hours of the phototherapy ($p< 0.05$). There was a non-significant change in lymphocyte subsets 48 hours after phototherapy ($p> 0.05$).

Our results do not go in agreement with **Neam & Zannoun[13]. investigated the influenc**e of phototherapy on some lymphocyte subsets and cytokine production in the prevention or treatment of neonatal hyperbilirubinemia. He found that the percentage of T lymphocyte subset was significantly lower in newborns at 72 hours of exposure to phototherapy.

Furthermore, in this study comparing the ratio between CD4/CD8 ratio among the studied groups and patients before and after 72hours of exposure to phototherapy, there was no statistical difference ($p>0.05$). These results agree with **Ped et al.** [12], who found no change in CD4 / CD8 ratio among patients or the studied groups.

Our results agree with **El Rashedy et al.** [9]**,** who reported that there is no statistically significant difference between lymphocytes subsets before and after 72 hours of exposure to phototherapy.

Our results are different from those of **Yahia et al.**[5], who reported that neonatal hyperbilirubinemia did not influence DNA damage and apoptosis in peripheral blood lymphocytes in full-term neonates. Also, our results disagree with**Karabayir et al.** [10]**,** who found no significant effects of hyperbilirubinemia on lymphocyte subgroups.

The result regarding infection follow-up shows there is no statistical difference in frequency of infection between the two groups in the first six months. This agrees with **Osman et al.** [14]**,** whostudied 30 full-term neonates with clinically significant indirect hyperbilirubinemia and followed them up over the admission period and 6 months after being discharged. They found substantial decreases in T and B lymphocyte % after exposure for phototherapy for 72 hours compared to their values before phototherapy.

In our study, regarding the relation between frequency of infection and CD: Only CD4 and CD8 were significantly higher among cases that had an infection (positive correlation between them).

In addition, in our study, the follow up of patients for six months after their discharge showed no increase in the frequency of infections or need for hospitalization. This does not agree with **Ustain et al.** [15], who studied the effect of neonatal jaundice and phototherapy on

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the frequency of first-year outpatient visits and found that there was a slight increase in first-year outpatient visits rates.

Conclusion:

Our study demonstrated that phototherapy did not affect either B and T cells or the occurrence or rate of infection and need for hospitalization. Lastly, we recommend more studies with more patients, and phototherapy only should be used if indicated.

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